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REVIEW

Wheat–barley hybridization: the last 40 years

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Abstract Several useful alien gene transfers have been reported from related species into wheat (*Triticum aestivum*), but very few publications have dealt with the development of wheat/barley (*Hordeum vulgare*) introgression lines. An overview is given here of wheat × barley hybridization over the last forty years, including the development of wheat × barley hybrids, and of addition and translocation lines with various barley cultivars. A short summary is also given of the wheat × barley hybrids produced with other *Hordeum* species. The meiotic pairing behaviour of wheat × barley hybrids is presented, with special regard to the detection of wheat–barley homoeologous pairing using the molecular cytogenetic technique GISH. The effect of in vitro multiplication on the genome composition of intergeneric hybrids is discussed, and the production and characterization of the latest wheat/barley translocation lines are presented. An overview of the agronomical traits (β-glucan content, earliness, salt tolerance, sprouting resistance, etc.) of the newly developed introgression lines is given. The exploitation and possible use of wheat/barley introgression lines for the most up-to-date molecular genetic studies (transcriptome analysis, sequencing of flow-sorted chromosomes) are also discussed.

Keywords Wheat × barley hybrids · Additions · Translocations · Introgressions · Homoeologous pairing

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Introduction

Wheat (*Triticum aestivum* L.) - alien hybridization makes it possible to transfer agronomically useful genes from one species to the other. Several useful alien gene transfers have been reported from wild species or rye (*Secale cereale* L.) into wheat, but very few publications have dealt with the development of wheat/barley (*Hordeum vulgare* L.) translocation lines. An overview is given here of wheat × barley hybridization over the last forty years, including the development and characterization of wheat/barley introgression lines in recent decades.

Production of wheat (*T. aestivum*) × barley (*H. vulgare*) hybrids, addition and substitution lines

Crosses between wheat and barley, two of the most important cultivated cereals, could make it possible to incorporate the earliness, favourable amino acid composition, salt and drought tolerance and good

tillering ability of barley into wheat. An even greater challenge would be the transfer of stem strength and winter hardiness from wheat into barley. Attempts to hybridize the two species began in the early 20th century, but the first demonstrably successful cross was reported by the Danish scientist Kruse in 1973. Encouraged by his success, attempts were made in several countries, aimed at producing new hybrids and progeny. Hybrids were developed at relatively greater frequency when barley was used as the female parent (Islam and Shepherd 1990). Barley × wheat hybrids were produced in numerous combinations by Islam et al. (1975), Fedak (1977), Thomas et al. (1977), Mujeeb-Kazi (1981), Clauss (1980), Shumny et al. (1981), Wojciechowska (1985) and Molnár-Láng et al. (1985). In crosses between a total of 18 barley varieties and 15 wheat varieties, the highest seed set was achieved when the wheat variety Chinese Spring (CS) was hybrized with the barley varieties Betzes and Ketch. A seed set of 15.4 % was reported by Islam et al. (1975), while Fedak (1980) achieved 49 % seed set, though only 2 % of the latter developed into plants. The Chinese Spring-Hope substitution line series was used to determine the chromosomal location of genes in Chinese Spring that permit crossability with Betzes barley (Fedak and Jui 1982). No progeny were obtained from substitution lines 5A, 5B, 5D, indicating these chromosomes of Chinese Spring, homoeologous group 5, are the major chromosomes responsible for permitting crossability with Betzes barley. All the hybrid plants obtained were raised in embryo culture, since the hybrid seeds have no endosperm and the embryos would die if left in the florets (Islam and Shepherd 1990). The barley × wheat hybrids exhibited complete male sterility, but when backcrossed with wheat it proved possible to produce BC_1 and BC_2 plants. The seed set in the first backcross was extremely low (0.5–1.2 seeds/spike) (Islam and Shepherd 1990). Due to the pistilloidy observed in the BC_1 and BC_2 plants the progeny remained sterile despite several backcrosses, making it impossible to develop fertile addition lines.

In order to eliminate pistilloidy, attempts were made to make the reciprocal cross, where wheat was the female and barley the male parent, but far fewer laboratories were able to report successful crosses and these involved a much smaller number of combinations (Islam and Shepherd 1990; Fedak 1980; Wojciechowska and Pudelska 1993; Molnár-Láng and Sutka 1994; Molnár-Láng et al. 2000b; Jauhar 1995; Taketa et al. 1998). It was found by Islam et al. (1978, 1981) that in this case, too, crosses between CS wheat and Betzes barley gave the highest seed set, but this was only 1.3 %, compared with 15.4 % for the reciprocal cross. In experiments carried out under optimum conditions in the Martonvásár phytotron, 3.3 % seed set was achieved with this combination (Molnár-Láng and Sutka 1994). When the barley variety Martonvásári 50 was crossed with CS wheat, several hybrid plants were obtained when barley was the female partner, but in the reciprocal combination, despite a very low seed set (0.18 %), no hybrid plants could be obtained (Molnár-Láng and Sutka 1994). A relatively short time after the development of the first wheat × barley hybrids, addition lines (2H, 3H, 4H, 5H, 6H, 7H) were also produced for the first time between CS wheat and the spring barley Betzes (Islam et al. 1978, 1981). By crossing this addition series with the relevant monosomic lines, substitution lines were developed by Islam and Shepherd (1992b, 1995) and Ya-Ping et al. (2003) for all the chromosomes except 1H and 5H.

Despite many attempts, it proved extremely difficult to expand the number of genotypes that could be successfully crossed, and very few hybrids were developed from genotypes with satisfactory agronomic traits (Wojciechowska and Pudelska 1993; Jauhar 1995; Taketa et al. 1998). It proved impossible to develop BC_1 seed on a substantial proportion of the new hybrids, so no fertile progeny could be obtained from the new combinations (Wojciechowska and Pudelska 1993; Jauhar 1995). The efficiency of hybrid development was greatly improved by Koba et al. (1991), who used the 2,4-D treatment that had been successfully applied in wheat × maize crosses. A number of Japanese wheat varieties were included in the crosses, among which Norin 12, Norin 61 and Shinchunaga gave better seed set than CS when pollinated with the barley variety Betzes. The highest seed set (8.25 %) was obtained from the Norin 12 × Betzes combination. F_1 hybrids could be produced from most of the embryos through embryo culture (Koba et al. 1991). Addition lines containing the barley chromosomes 5H and 6H were developed from a cross between the wheat variety Shinchunaga and the barley variety Nyugoruden, and translocation lines were produced containing the 5HS.5BL translocation chromosome pair in addition to 42 wheat

chromosomes (Koba et al. 1997). Backcross progenies (BC_1 and BC_2) were developed from the combination of wheat cv. Shinchunaga × barley line T3-7aai by Malysheva et al. (2003) in Germany. The genome composition of the backcross progenies was analysed using genomic in situ hybridization (GISH) and microsatellite markers. Some of the barley chromosomes (2H, 4H) were entirely eliminated from the BC_2 plants, the presence of 1H caused sterility, and chromosome segments from other barley chromosomes (3H, 5H, 6H, 7H) were detected in some BC_2 plants. The development of disomic addition lines from this combination was not reported.

Barley has great genetic diversity for many agronomically important traits (spring or winter habit, two-rowed or six-rowed, tolerance to abiotic stresses, yield ability, earliness, quality, adaptation, etc.). In order to utilise the useful agronomic traits of barley cultivars it would be worth producing wheat/barley addition and introgression lines with agronomically adaptable winter barley cultivars. A series of two new additions was reported from the wheat × barley hybrids produced with winter barley cultivars in Martonvásár (Molnár-Láng et al. 2000b). Backcross progenies were developed on the hybrids at very low frequency (Molnár-Láng et al. 2000b, 2005). Wheat–barley disomic addition lines (2H, 3H, 4H, 6HS, 7H, 1HS isochromosome) were produced and identified using molecular cytogenetic methods from hybrids between winter wheat line Mv9kr1 and the German two-rowed winter barley Igri (Szakács and Molnár-Láng 2007, 2010). In order to increase the allelic variation in wheat/barley introgressions, new wheat/barley disomic addition lines were developed containing the 2H, 3H, 4H, 6H and 7H chromosomes of the six-rowed Ukrainian winter barley cultivar Manas (Molnár-Láng et al. 2012). This cultivar is agronomically much better adapted to Central European environmental conditions than the two-rowed spring barley cultivar Betzes previously used.

Wheat × barley hybrids produced with other *Hordeum* species

In addition to cultivated hexaploid wheat (*T. aestivum* L.) and cultivated barley (*Hordeum vulgare* L.), hybrids have also been developed between other *Triticum* and *Hordeum* species, the most successful of which is hexaploid tritordeum, which arose from a cross between *Triticum turgidum* L. ssp. *durum* (Desf.) Husn. (synonym: *T. durum*) and *Hordeum chilense* Roem. et Schulz. (Martín and Sanchez-Monge Laguna 1980, 1982). *H. chilense* was previously pollinated with hexaploid *T. aestivum* to produce an F_1 hybrid (Martín and Chapman 1977), from which a partially fertile amphidiploid was produced by means of colchicine treatment (Chapman and Miller 1978). The amphidiploid was then backcrossed to wheat to develop wheat/*H. chilense* addition lines (Miller et al. 1981). Later, *H. chilense* was pollinated with *Triticum durum*, after which the hybrid was treated with colchicine to develop fertile amphidiploids with 42 chromosomes (Martín and Sanchez-Monge Laguna 1980, 1982). As this new amphidiploid exhibited fewer meiotic chromosome pairing anomalies and fertility problems than the primary triticale, it was assumed that, like triticale, it could be cultivated as a new plant species, and was named tritordeum. Hexaploid tritordeum was found to have a protein content of 19–24 % (Martín and Cubero 1981), so numerous analyses were made to provide a detailed description of the quality parameters of the new species. After 6–7 years of self-fertilisation in field experiments, it was established that the new species yielded only 20–40 % as much as cultivated wheat, but had a protein content amounting to 17.6–25.2 % of the dry matter (Cubero et al. 1986). Its other quality parameters (fibre, lignin, cellulose and hemicellulose contents, amino acid composition) were similar to those of cultivated wheat. A multi-location study under varying growth conditions revealed important information about the effect of water availability on the yield of tritordeum. In the lowest yielding environments tritordeum and triticale had similar yields (Villegas et al. 2010). However, under better growth conditions tritordeum was found to yield less than wheat and triticale. It is suggested that tritordeum could be a new option for cultivation in very dry environments.

In the course of cytological analyses, the chromosome number and chromosome pairing of the new species were first monitored using the Feulgen method, which revealed a high level of chromosome stability (Martín and Cubero 1981). Later the *H. chilense* chromosomes were studied by means of C-banding (Fernandez et al. 1985) and fluorescence in situ hybridization (FISH) using repetitive DNA sequences (Cabrera et al. 1995). Hybridization with

the pAs1 DNA clone isolated from *Aegilops squarrosa* (synonym: *Ae. tauschii* Coss.) gave a hybridization pattern similar to that of the D genome chromosomes of wheat for the *H. chilense* chromosomes, with strong hybridization signals on the telomeres. The chromosomes of *Hordeum vulgare* cannot be identified with this probe, as it gives diffuse signals on barley. The hybridization pattern obtained with C-banding bore more resemblance to that of the wheat chromosomes and strong telomeric bands were observed on the *H. chilense* chromosomes, while in the case of *H. vulgare* chromosomes, C-banding revealed interstitial bands near the centromere (Cabrera et al. 1995). Molecular cytogenetic analysis showed that *H. chilense* was genetically distant from cultivated barley. Numerous papers have been published on the taxonomical classification of *Hordeum* species (Löve 1982, 1984; Dewey 1984), which were first classified on the basis of morphological observations, then on the basis of chromosome pairing in interspecific hybrids, and later in terms of the conclusions drawn from molecular genetic analysis. Bothmer et al. (1986, 1987) used the data of chromosome pairing analysis to divide the *Hordeum* species into four basic genomes (I, Y, X and H). Molecular genetic analysis later confirmed this classification (Svitashev et al. 1994), showing that *H. vulgare* and *H. bulbosum* contained genome I and *H. murinum* genome Y, while *H. chilense* was one of the species carrying the H genome, and *H. marinum* Huds. had an X genome. This classification confirmed the relatively distant relationship between *H. vulgare* and *H. chilense*.

In an effort to improve the agronomic traits of tritordeum, further crosses were made, primarily with triticale. The progeny were then analysed using various cytogenetic methods (Fernandez-Escobar and Martín 1985; Lima-Brito et al. 1996). The chromosomes of both *H. chilense* and rye could be identified by means of FISH (Lima-Brito et al. 1996). The hexaploid tritordeum was also crossed with *H. vulgare*, but the amphidiploid developed by treating the F_1 hybrid with colchicine proved to be sterile (Martín et al. 1995). Transgenic lines were developed by transforming tritordeum (Barcelo et al. 1994) and these were later studied in nursery experiments (Hernandez et al. 2001). A new cytoplasmic male sterility (CMS) source designated msH1 has been reported in bread wheat by Martín et al. (2009). This system uses the cytoplasm of *H. chilense*. The male sterility of alloplasmic wheat containing *H. chilense* cytoplasm is stable under various environmental conditions and the plants exhibit no developmental or floral abnormalities, except for slightly reduced height and some delay in heading. There is thus real potential for the development of a viable technology for hybrid wheat production. The addition of chromosome $6H^{ch}S$ from *H. chilense* accession H1 was able to restore the pollen fertility of the CMS phenotype induced by the presence of *H. chilense* cytoplasm in wheat. An optimal combination for fertility restoration was the translocation $T6H^{ch}.6DL$, developed by Martín et al. (2009).

In addition to *Hordeum chilense*, the following *Hordeum* species have been hybridised with wheat:

- *H. spontaneum* [syn.: *H. vulgare* ssp. *spontaneum* (C. Koch) Thell] (Islam and Shepherd 1990; Taketa et al. 1995),
- *H. bulbosum* L. (Barclay 1975; Blanco et al. 1986),
- *H. bogdanii* Wil. (Kimber and Sallee 1976),
- *H. pussillum* Nutt. (Finch and Bennett 1980),
- *H. geniculatum* All. (Clauss 1983; Pershina et al. 1988),
- *H. pubiflorum* Hook. f. (Fedak 1983),
- *H. californicum* Covas & Stebbins [syn.: *H. brachyantherum* Nevski ssp. *californicum* (Covas & Stebbins)] (Gupta and Fedak 1985),
- *H. marinum* Huds. (Jiang and Dajun 1987; Islam et al. 2007; Pershina et al. 2009),
- *H. depressum* (Scribn. & Smith) Rydb. (Jiang and Dajun 1987).

A complete set of wheat–wild barley (*Hordeum vulgare* ssp. *spontaneum)* chromosome addition lines was developed by Taketa and Takeda (2001). The chromosome constitution of the addition lines was confirmed by C-banding and GISH hybridization. Addition lines for the entire 1H chromosome and its long arm are only available as monosomic and monotelosomic additions, respectively, because of sterility. Disomic additions involving individual chromosomes of sea barleygrass (*Hordeum marinum* Huds.) in CS wheat were obtained by Islam and Colmer (2008). The salt tolerance of the wheat–*H. marinum* amphiploid was intermediate to that of its parents (Islam et al. 2007). Alloplasmic wheat–barley substitution and addition lines were produced by Pershina et al. (2009) from *H. marinum* ssp. *gussoneanum* Huds. × *T. aestivum* hybrids.

In vitro multiplication of wheat × barley hybrids

Sterile interspecific and intergeneric hybrids can be maintained and multiplied in a vegetative manner through callus formation in tissue culture (in vitro). Interspecific and intergeneric hybrids produced in wide crosses are often not only male sterile, but also have such a low level of female fertility that seeds are only set at extremely low frequency even when pollinated with one of the parents. Tissue culture makes it possible to multiply hybrids that can only be developed at very low frequency, thus producing enough progeny for backcrossing. The in vitro multiplication of interspecific and intergeneric hybrids developed between cereal species has been reported for various combinations: barley × rye (Shumny and Pershina 1979); wheat × rye (Armstrong et al. 1983; Doré et al. 1988); *Aegilops crassa (Triticum crassum)* × *Hordeum vulgare* (Nakamura et al. 1981); wheat × *Agropyron* hybrids (Sharma et al. 1984; Bai and Knott 1993); *Elymus canadensis* L. × *Psathyrostachys juncea* (Fisch.) Nevski (Park et al. 1990). When the progeny were subjected to cytological analysis, deviations were observed in all cases compared with the initial hybrids. It was established that the somaclonal variability observed during the in vitro multiplication of plants (Larkin and Scowcroft 1981) could lead to useful rearrangements during the maintenance of interspecific and intergeneric hybrids in tissue culture (Fedak 1985). Amphidiploids with a doubled chromosome number have been successfully produced from F_1 hybrids (Doré et al. 1988; Ter Kuile et al. 1988), translocations have been observed (Sharma et al. 1984) and in some cases the regenerants have been found to have increased fertility (Sharma et al. 1984; Fedak and Grainger 1986; Molnár-Láng et al. 1991).

Wheat–barley hybrids were multiplied in tissue culture by Pershina and Shumny (1981), Chu et al. (1984), Junming et al. (1985), Galiba et al. (1986), Surikov and Kissel (1988) and Koba et al. (1988). Detailed cytological analyses on the regenerants were published by Junming et al. (1985), Fedak and Grainger (1986), Shimada et al. (1987) Fedak et al. (1987) and Molnár-Láng et al. (1991). Chromosome numbers differing from that of the initial hybrid (28) were observed by Junming et al. (1985) and Koba et al. (1988) in some regenerants (21–27) and all the authors recorded the occurrence of amphidiploid cells. The appearance of telocentric chromosomes in the regenerants was observed in several experiments (Junming et al. 1985; Koba et al. 1988; Molnár-Láng et al. 1991). A detailed analysis was made of the meiosis of regenerant hybrids by Molnár-Láng et al. (1991), who found an increase in the rate of homoeologous chromosome pairing. A similar conclusion was drawn by Dahleen (1999) when investigating the progeny regenerated from barley × wild rye hybrids in tissue culture. As no backcross seeds were obtained from the initial hybrid of facultative wheat cv. Asakaze × winter barley cv. Manas, young inflorescences of the hybrids were used for in vitro multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each in vitro multiplication cycle (Molnár-Láng et al. 2005). The seven barley chromosomes were present even after the third cycle but abnormalities were observed. Chromosome breakages occurred; the number of barley telocentrics became significantly higher after the third cycle and amphidiploid cells with 56 chromosomes were counted. The number of wheat–barley chromosome arm associations, i.e. homoeologous pairing frequency increased after in vitro multiplication (Molnár-Láng et al. 2005).

Chromosome pairing in wheat × barley hybrids

The meiotic pairing behaviour of wheat × barley hybrids was first analysed with the Feulgen method by several scientists. Islam and Shepherd (1980) observed 28 univalent chromosomes in the meiosis of wheat × barley hybrids in the majority of the cells, though in a few cells chromosome pairing could be seen, with an average of 0.7 bivalents per pollen mother cell. A higher rate of chromosome pairing was recorded by Fedak (1977) in the meiosis of barley × wheat hybrids, resulting in a chiasma frequency of 1.82 per pollen mother cell. This was higher than the rate reported earlier in wheat haploids (Riley and Law 1965), suggesting that pairing also took place between barley and wheat chromosomes. Fedak (1977) drew attention to the phenomenon of homoeologous pairing between the chromosomes of two distantly related genera, and suggested that this should be confirmed with the Giemsa technique, the best method available at the time. Later Jauhar (1995) demonstrated a

chiasma frequency of 2.16–6.72 per pollen mother cell in wheat × barley hybrids developed using the barley variety Luther. These data pointed to pairing between wheat and barley chromosomes, but as the chromosomes were analysed in meiosis using the Feulgen method, it was not possible to identify the individual chromosomes. An average of 5.03–6.63 bivalents per pollen mother cell could be observed in wheat × barley hybrids produced using the *Ph* mutant of CS, together with a small number of trivalents and quadrivalents (Sethi et al. 1986), but pairing between wheat and barley could not be demonstrated with the Feulgen method. Wojciechowska (1985) performed detailed meiosis analysis in several barley × wheat hybrid combinations and found a chiasma frequency of 1.17–1.98 per pollen mother cell in hybrid cells containing 28 chromosomes.

Islam and Shepherd (1988) elaborated a method for the detection of pairing between wheat and barley chromosomes. They crossed ditelosomic wheat/barley addition lines with a high-pairing strain of an *Ae. speltoides* genotype carrying the *Ph* suppressor gene. F_1 hybrids possessing 28 + 1 telocentric somatic chromosomes (21 wheat + 7 *Ae. speltoides* plus 1 barley telocentric) were grown. Pairing between telocentric and non-telocentric chromosomes was observed in 1.2–4.5 % of the pollen mother cells. Triple monosomic addition lines were developed in a wheat monosomic background, one of which contained 19 pairs of wheat chromosomes together with one 5B *Ph* mutant chromosome, one 3HL barley chromosome arm and a 3A wheat chromosome (Islam and Shepherd 1992a). In another line the 5B *Ph* mutant was accompanied by one 6HS and one 6B chromosome. In the triple monosomic addition lines, plants carrying the 3HL and 6HS barley chromosome arms only exhibited pairing in 0.3–0.7 % of the cells. These experiments proved that chromosomes of the distantly related species wheat and barley are capable of pairing with each other, thus allowing recombinations to occur.

The meiotic instability of wheat × barley hybrids was noted by a number of authors, who found many cells with hypo- or hyperploid chromosome numbers in addition to cells with 28 chromosomes (Fedak 1980; Mujeeb-Kazi and Rodriguez 1983; Islam and Shepherd 1980; Wojciechowska 1985). Islam and Shepherd (1980) observed that the chromosome number became doubled in some cells during meiosis (restitution nuclei). In these hybrids the univalent chromosomes assembled in the equatorial plate during metaphase I of meiosis, but in many cells, instead of migrating to the two poles in anaphase I, the chromosomes remained together, forming a chromatin mass, thus leading to the formation of cells with a doubled chromosome number. In these cells, however, it was often observed that the second phase of meiosis did not take place, preventing the development of microspores with the full chromosome complement, which would restore the fertility of the hybrids (Islam and Shepherd 1980). It can be assumed that the egg-cells which became fertilised and set seed when the hybrids were backcrossed arose from megaspores with a doubled chromosome number.

Wheat–barley chromosome pairing was first detected using GISH by Molnár-Láng et al. (2000b). Meiotic analysis of the wheat × barley hybrid Mv9 kr1 × Igri revealed 1.59 chromosome arm associations per cell using the Feulgen method (Molnár-Láng et al. 2000b). The number of chromosome arm associations increased to 4.72 after in vitro culture. According to GISH analysis, wheat–barley chromosome arm associations made up 3.6 % of the total in the initial hybrid and 16.5 % of the total in progenies of the Mv9 kr1 × Igri hybrids regenerated in vitro. The meiotic pairing behaviour of a wheat–winter barley hybrid (Asakaze × Manas) was analysed using GISH after long-term maintenance in tissue culture (Molnár-Láng et al. 2005) (Fig. 1). As no backcross seeds were obtained from the initial hybrid, young inflorescences of the hybrids were used for in vitro multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each in vitro multiplication cycle. The number of wheat–barley chromosome arm associations increased after the second and third cycles. Amphidiploid cells containing seven barley bivalents were counted after the third cycle. The use of the GISH technique to demonstrate wheat–barley chromosome pairing in the hybrids, and especially in their in vitro-regenerated progenies, proved the possibility of producing recombinants between these two genera, and thus of transferring useful characters from barley into wheat (Molnár-Láng et al. 2000b, 2005). In some regenerants in vitro conditions caused an increase in chromosome arm association frequency and in fertility.

Production of wheat/barley recombination and translocation lines

Islam and Shepherd (1992a) were the first to develop recombinations from wheat × barley crosses. Triple monosomics were developed from crosses between wheat/barley ditelosomic substitution lines and the *Ph* mutant of CS wheat. In addition to 19 wheat chromosome pairs, the triple monosomic additions contained one barley telocentric chromosome, the homoeologous wheat chromosome and one 5B *Ph* mutant chromosome. With this method wheat/barley recombinations involving six 6HL and six 3HL chromosome segments were detected among the progeny. The presence of the recombinations was proved by isoenzyme analysis: the progeny were found to contain isoenzymes located either on the 6A and 6H or on the 3A and 3H chromosomes. Sherman et al. (2001) also utilised the effect of the *Ph* mutant gene to develop recombinations from the 4H and 5H wheat/barley addition lines produced by Islam et al. (1981). The presence of the recombinations was confirmed with PCR-based molecular markers. The use of GISH to detect the occurrence of wheat–barley translocations was first reported by Schwarzacher et al. (1992). The translocation line was developed by Islam and Shepherd (unpublished data) and isoenzyme analysis proved that at least the segment of the 4HL barley chromosome arm carrying the gene coding for the barley β-amylase isoenzyme had been incorporated into this line. GISH analysis then demonstrated that the whole of the 4HL chromosome arm was present in the translocation line, i.e. a centric fusion had occurred between wheat and barley. The occurrence of spontaneous translocations was observed by Koba et al. (1997) in the progeny of a cross between Shinchunaga wheat and Nyugoruden barley. The translocation chromosome was identified with C-banding and, using an earlier nomenclature, it was found that it involved the short arm of chromosome 7 of barley and the long arm of wheat chromosome 5B. When the names of the barley chromosomes were later revised, the old chromosome 7 was renamed 5H (Linde-Laursen et al. 1997) and it became clear that a homoeologous translocation had indeed taken place.

Various methods are available for producing translocations, including irradiation (Sears 1956, Szakács et al. 2010) or the induction of homoeologous pairing (Riley and Chapman 1958; Sears 1972; Griffiths et al. 2006). A number of genes from common wheat promote chromosome pairing and several act as inhibitors (Sears 1976). The pairing homoeologous gene, *Ph1*, on the long arm of chromosome 5B, has the most decisive effect. In its presence, pairing is restricted to homologues; in its absence, homoeologues also pair, albeit less frequently than homologues. The simple deletion of *Ph1*, or the counteraction of its effect by high-pairing types of *Ae. speltoides* or *Ae. mutica*, can induce many Triticinae chromosomes to pair with their wheat homoeologues. Such induced homoeologous pairing is usually the method of choice for transferring genes from alien chromosomes to those of wheat. The "*Ph* system" was used by Islam and Shepherd (1992a) and by Sherman et al. (2001) to produce wheat–barley translocations. A unique genetic system exists in common wheat, which induces frequent chromosomal structural rearrangements. This system, the gametocidal (Gc) system, involves alien chromosomes called Gc chromosomes, which were introduced into common wheat from certain wild species belonging to the genus *Aegilops*. This system proved to be effective in inducing structural rearrangements in the barley chromosomes added to common wheat, as well as in common wheat chromosomes (Endo 2009). The rearranged chromosomes thus induced include deletions of barley chromosomes and translocations between the barley and wheat chromosomes. Lines carrying rearranged barley chromosomes are designated as 'dissection lines' (Endo 2009). Schubert et al. (1998) developed wheat–barley translocations from wheat/barley disomic addition lines by exploiting the gametocidal effect of the 2C chromosome of *Aegilops cylindrica*. The 7H wheat/barley addition line was crossed with the 2C wheat/*Ae. cylindrica* addition line and the resulting line, containing two different alien chromosomes, was self-fertilised. Lines carrying barley deletions and wheat–barley translocations were selected from the progeny. More than ten translocation lines carrying segments of the 7H barley chromosome were produced. The incorporation of the barley chromosome segments was detected by means of GISH, and with FISH using the repetitive probe HvT01. These 7H deletion and translocation lines were then used for the physical mapping of the 7H barley chromosome (Serizawa et al. 2001). Nasuda et al. (2005), Ashida et al. (2007) and Sakai et al. (2009) performed deletion mapping on barley chromosomes 7H, 5H and 3H using barley dissection lines and barley-specific EST

markers. The barley dissection lines were produced from CS-Betzes addition lines, so they all carried chromosome segments from Betzes barley.

Molnár-Láng et al. (2000a) developed translocations from wheat–barley hybrids regenerated in tissue culture, using GISH for confirmation. The origin of the barley chromosome segments involved in the selected homozygous translocation lines was determined using molecular markers (Nagy et al. 2002). Segments of various sizes from the 1H, 3H, 4H and 5H chromosomes were found to have been incorporated in the translocation lines. These lines were then used for the physical mapping of microsatellite markers previously located on the barley chromosomes. Sepsi et al. (2006) produced wheat/barley translocations as the result of induced homoeologous chromosome pairing in a 4H(4D) wheat–barley substitution line by crossing with the line CO4-1, which carries the *Ph* suppressor gene from *Aegilops speltoides*. Kruppa et al. (2013) reported the development of a 4HL.5DL Robertsonian translocation line after crossing the 4H(4D) wheat–barley substitution line with the CS*ph1b* mutant. The rearrangement was confirmed with sequential GISH, FISH and SSR markers. A spontaneous wheat–barley translocation was identified using sequential GISH, FISH and SSR markers by Cseh et al. (2011) in the progenies of the Asakaze × Manas hybrid. This translocation line carries a 4BS wheat chromosome arm and a 7HL chromosome arm from the Ukrainian six-rowed winter barley. Another spontaneous wheat/barley translocation line was identified as 5HS-7DS.7DL in the progenies of the Mv9kr1 × Igri wheat–barley hybrid (Kruppa et al. 2013). Despite the non-compensating nature of the translocation, the plants showed good viability. Of the 45 microsatellite markers analysed, ten failed to amplify any 7DS-specific fragments, signalling the elimination of a short chromosome segment in the telomeric region. The breakpoint of the 5HS-7DS.7DL translocation appeared to be more distal than that of reported deletion lines, thus providing a new physical landmark for future deletion mapping studies.

Characterization and exploitation of wheat–barley introgression lines

Alien additions are primarily produced to add specific desirable genes to a crop species (Gale and Miller 1987), but addition lines can be used for many other purposes, such as mapping genes and markers on introgressed alien chromosomes, examining alien gene regulation, understanding meiotic pairing behaviour and chromosome structure, and isolating individual chromosomes and genes of interest (Chang and de Jong 2005; Cho et al. 2006). The wheat–barley addition lines produced in various cultivar combinations (CS × Betzes, Mv9 kr1 × Igri, Asakaze × Manas) had several morphological traits in common (Molnár-Láng et al. 2012). The 4H additions had the best fertility and 7H the lowest in all three combinations. The 2H addition line had a lax spike structure in every cultivar combination. The 3H addition had the shortest, most compact spike of all the addition lines in the Mv9kr1-Igri and CS-Betzes sets. The 3H Asakaze–Manas addition also had a short spike, but it was not as dense as in the other two combinations. This addition line showed a high level of genetic instability, which cannot yet be explained. The 4H addition line had the tallest plants and 3H the shortest. The 6H and 7H additions were shorter than the 4H, as also observed in the Mv9kr1-Igri addition lines. Unfortunately the 5H addition could not be selected from the Mv9 kr1 × Igri and Asakaze × Manas combinations, as this chromosome was eliminated most frequently from the backcross progenies (Molnár-Láng et al. 2005). Barley chromosome 1H caused sterility even in the presence of other barley chromosomes such as 2H, 3HS, 4H, 5HS and 7H. A fertile addition line involving the entire barley chromosome 1H could not be produced by Islam et al. (1978) because a gene on the long arm of this chromosome caused sterility when present in a wheat background. The double monosomic 1H and 6H addition became partly female fertile and a few backcross seeds were produced after pollinating them with normal wheat (Islam and Shepherd 1990). Plants disomic for 6H and monosomic for 1H were developed, which had some self-fertility (Islam and Shepherd 2000). Unfortunately none of the BC_2 plants from the Asakaze × Manas and Mv9 kr1 × Igri combinations carried the barley chromosomes 6H and 1H together.

Hart et al. (1980) used differences between wheat and barley isozymes to determine the chromosomal locations of barley structural genes for these isozymes. Genes controlling more than 58 isozymes have been allocated to specific barley chromosomes or to arms

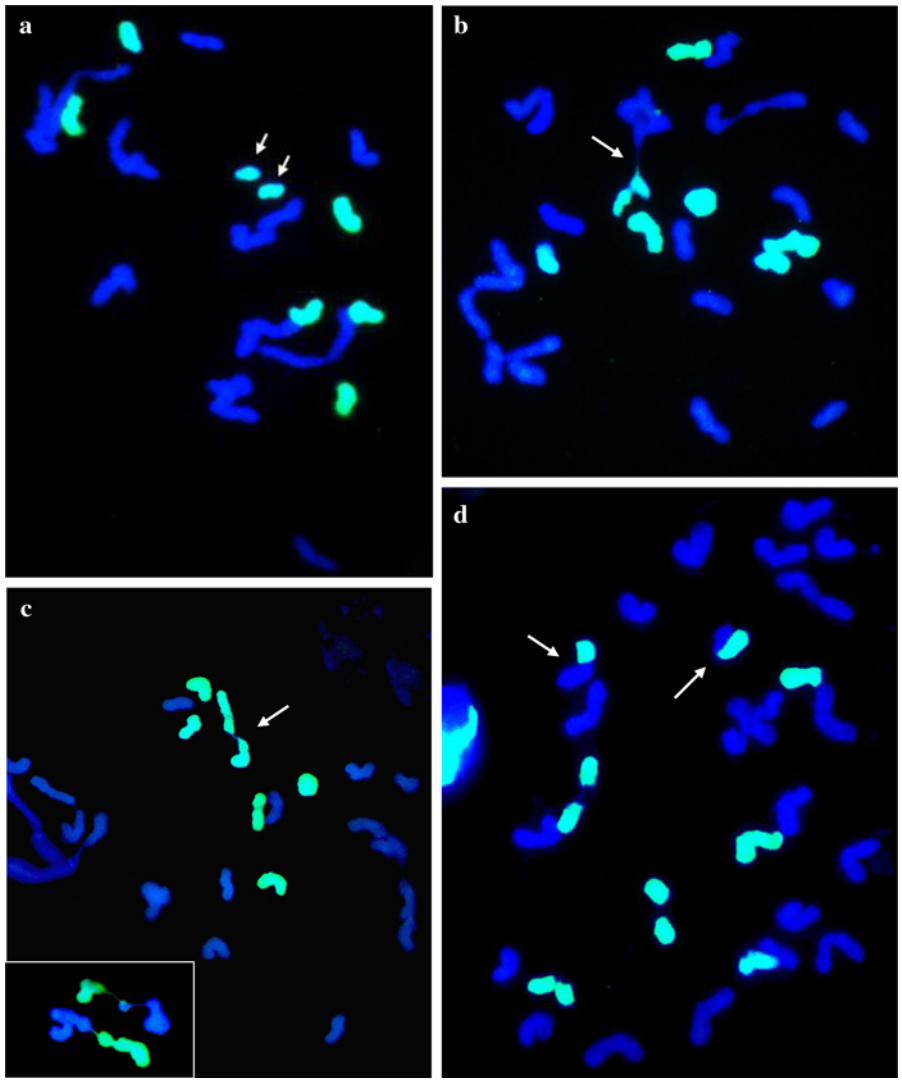

Fig. 1 GISH on meiotic chromosomes (**a–d**) of wheat–barley (Asakaze-Manas) hybrids multiplied in vitro. Total barley genomic DNA was labelled with Fluorogreen and used as a probe. Wheat chromosomes counterstained with DAPI. **a** Six barley univalents and two misdivided barley chromosomes (*arrows*). **b** A wheat ring bivalent pairing with a barley chromosome making a trivalent configuration (*arrow*). **c** Five barley univalents and a barley–barley chromosome pairing (*arrows*). Two wheat–barley bivalents (*part* of another cell, *small box*). **d** Two wheat–barley translocations (*arrows*), four barley univalents and two misdivided chromosomes

thereof using the wheat–barley addition lines (Islam and Shepherd 1990). The effect of the added barley chromosomes on heading characters was studied by Murai et al. (1997) using the CS-Betzes addition lines produced by Islam et al. (1978) together with the 5H and 6H Shinchunaga/New Golden additions produced by Koba et al. (1997). The earliest flowering was observed on the CS-Betzes 5H addition line and on the Shinchunaga/New Golden 5H addition lines. Murai et al. (1997) demonstrated that the heading characters of wheat can be altered by barley genes. The Mv9kr1-Igri and Asakaze–Manas wheat/winter barley addition lines made it possible to study the effects of chromosomes from winter barley cultivars on flowering time in the wheat genetic background under various environmental conditions (Farkas et al. 2013). The winter barley chromosome additions significantly influenced the flowering time of wheat both in a

controlled environment test and under field-sown conditions. Unfortunately the 5H addition was missing from both combinations, because 5H was the first chromosome to be eliminated from the backcross derivatives (Molnár-Láng et al. 2005, 2012; Szakács and Molnár-Láng 2010). Of all the barley addition lines, the effect of the 4H and 7H additions was the most characteristic. The 7H addition lines were the earliest in both cultivar combinations in each treatment. In the Mv9kr1-Igri combination the 4H addition was the latest under all the environmental conditions. In the Asakaze–Manas combination the 4H addition was the latest under short-day and long-day illumination in the phytotron, but the 6H addition was the latest without vernalisation and in the field in 2012. There was 12 and 11 days' difference between the flowering times of the 7H and 4H Mv9kr1-Igri and Asakaze–Manas addition lines in the field in 2012, which increased to 52 and 44 days under short-day illumination in the phytotron (Farkas et al. 2013). Only two days' difference was observed between the CS-Betzes 7H and 4H addition lines by Murai et al. (1997) under short-day illumination in the phytotron, which could be primarily due to the fact that Betzes, being a spring barley, did not carry the ZCCT-H genes at the VRN-H2 locus, giving further indirect proof that the effect of Vrn-H2 was detected in these addition lines.

The dietary fibre (1,3;1,4)-β-D-glucan (β-glucan), is a major quality parameter of cereals. Barley β-glucans are beneficial to human health, as they are a major source of soluble dietary fibre and have been recognized both as potential cholesterol-lowering polysaccharides (Kerckhoffs et al. 2003) and as non-specific immune-activators (Allendorf et al. 2005). The grain of barley is one of the most important β-glucan sources having a β-glucan content ten times higher than that of wheat. The cellulose synthase-like F6 (*CslF6*) gene, encoding a putative β-glucan synthase, has been assigned to the 7H chromosome (Burton et al. 2008). The presence of the *HvCslF6* gene, responsible for (1,3;1,4)-β-D-glucan production, was revealed in the centromeric region of 7HL using the 4BS.7HL Asakaze–Manas translocation line (Cseh et al. 2011). An increased (1,3;1,4)-β-D-glucan level was also detected in the translocation line, demonstrating that the *HvCslF6* gene is of potential relevance for the manipulation of wheat (1,3;1,4)-β-D-glucan levels. The Mv9kr1-Igri 1HS ditelosomic and Mv9kr1-Igri 7H disomic wheat/barley addition lines carrying the *HvCslF9* and *HvCslF6* barley genes, respectively, were used to investigate the additive effect of barley cellulose synthase-like genes on the wheat β-glucan content (Cseh et al. 2013). A significantly higher β-glucan level was detected in the leaves and grains of the wheat/barley 1HS and 7H addition lines compared to the control wheat line. The expression of the *HvCslF9* and *HvCslF6* genes in the genetic background of wheat was also determined by quantitative RT-PCR, and the *HvCslF9* gene was mapped to the short arm of the 1H chromosome (Cseh et al. 2013). The *HvGlb1* barley gene, encoding (1,3;1,4)-β-D-glucan endohydrolase isoenzyme EI, is possibly involved in the regulation of the β-glucan level during grain development. Previously this was also mapped to the barley 1H chromosome, and this study made it clear that it was located on the 1HL chromosome arm. Zou et al. (2012) recently identified wheat–barley 2HL chromosome translocation lines derived from crosses between CS-Betzes 2H disomic substitution lines and Chinese wheat varieties. These translocations carry the *Isa* gene encoding the barley bifunctional α-amylase/subtilisin inhibitor (BASI). Because BASI is more effective in inhibiting wheat AMY2 than the α-amylase inhibitors of other cereals (Henry et al. 1992), the introduction of the barley *Isa* gene into wheat may regulate endogenous α-amylase activity during starch granule synthesis in the developing grain and reduce the level of preharvest sprouting damage.

Wheat–barley chromosome addition lines are useful genetic resources for studying the transcript accumulation patterns of barley in a wheat genetic background and for the large-scale physical mapping of genes. In a study performed by Cho et al. (2006) the CS-Betzes addition lines were examined with the Barley1 Affymetrix GeneChip probe array and a total of 1,787 barley transcripts were detected and physically mapped to barley chromosomes and to the long and short arms of chromosome 6H. The same method and plant materials were used to physically map barley genes to their respective chromosome arm locations by Bilgic et al. (2007), who mapped 1,257 barley genes to chromosome arms 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS and 7HL. The number of genes assigned to individual chromosome arms ranged from 24 to 197.

Flow sorting can be effective for isolating large samples of alien chromosomes from metaphase suspensions if the flow karyograms of sorted additions

demonstrate distinct peaks not present in those of the parental species (Suchánková et al. 2006). The telocentric chromosomes of Betzes barley were isolated from the CS-Betzes ditelosomic addition lines, thus allowing the barley genome to be dissected into fractions each representing only about 6–12 % of the total genome (Suchánková et al. 2006). The DNA of flow-sorted chromosomes can be used for the isolation of molecular markers, for physical mapping using PCR and FISH, for the integration of genetic and physical maps and for the construction of chromosome-specific DNA libraries, including sequences cloned in bacterial artificial chromosome vectors. The first barley chromosome to be isolated by flow sorting and shotgun sequencing was 1H (Mayer et al. 2009). As there is no significant difference in the size of the barley chromosomes, the other six chromosomes could only be sorted from wheat/barley ditelosomic addition lines, in spite of that some barley chromosomes are identifiable based on morphology.

Twelve barley chromosome arms (2HS to 7HL) were purified separately by flow cytometry (Suchánková et al. 2006), after which the DNA was amplified by multiple displacement amplification (MDA) and then shotgun sequenced (Mayer et al. 2011). Using this procedure, between 2,261 and 3,616 genes were tentatively positioned along each of the individual barley chromosomes, representing a cumulative set of 21,766 genes across the entire barley genome. An additional set of 5,815 genes could not be integrated into the genome zippers based on conserved synteny models, but were associated with individual chromosomes/chromosome arms. Overall, it was possible to tentatively position 27,581 barley genes, or 86 % of the estimated 32,000 gene repertoire of the barley genome, into chromosomal regions (Mayer et al. 2011). Among the 21,766 genes anchored to the genome zipper, 3,125 (14 %) genes were allocated to the genetic centromeres. Based on the 454 sequence- and array-based gene assignments to chromosome arms, all but nine of these 3,125 genes were distributed to specific arms of chromosomes 1H to 7H.

From the practical point of view new wheat–barley hybrids need to be produced using a wider range of barley genotypes carrying genes responsible for useful agronomic traits (e.g. drought tolerance, high β-glucan content, salt tolerance, earliness). The major limitation for successful gene transfer from barley into wheat is the low crossability between these species. The efficiency with which wheat × barley hybrids can be produced should be increased by hormone treatment at pollination, and by improving the yield of embryo culture. Among the various methods available for producing translocations from wheat × barley hybrids and additions, the gametocidal system is currently the most promising. Although Tritordeum already exists as the product of the wheat × *H. chilense* combination, and fertile amphiploids have been produced with *H. marinum* and *H. californicum,* fertile *T. aestivum* × *H. vulgare* amphiploids have not yet been developed. Unfortunately the chromosome 1H of *H. vulgare* carries a gene (*Shw*) that causes sterility in wheat background (Taketa et al. 2002) and it prevents the production of a fertile amphiploid. The development of small barley introgressions in the wheat genetic background has been started, but a much larger quantity of translocations carrying genes responsible for useful traits are needed. Wheat/barley translocations are ideal material for the physical mapping of wheat and barley chromosomes, as the two genomes can be clearly identified using GISH, and the physical landmarks can be used in genome mapping.

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